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Alfachment

ALIGNMENTS

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RESULT 1
US-08-632-470-53
; Sequence 53, Application US/08632470
 Patent No. 5976791
GENERAL INFORMATION:
    APPLICANT: MABILAT, CLAUDE
    APPLICANT:
                RAOULT, DIDIER
                        NUCLEOTIDE FRAGMENTS CAPABLE OF
    TITLE OF INVENTION:
                        HYBRIDIZING SPECIFICALLY TO RICKETTSIA RDNA OR RRNA AND
    TITLE OF INVENTION:
    TITLE OF INVENTION:
                        THEIR USE AS PROBES OR PRIMERS
    NUMBER OF SEQUENCES:
    CORRESPONDENCE ADDRESS:
      ADDRESSEE: OLIFF & BERRIDGE
       STREET: P.O. BOX 19928
      CITY: ALEXANDRIA
STATE: VA
COUNTRY: USA
      ZIP: 22320
    COMPUTER READABLE FORM:
      MEDIUM TYPE: Floppy disk
       COMPUTER: IBM PC compatible
      OPERATING SYSTEM: PC-DOS/MS-DOS
SOFTWARE: PatentIn Release #1.0, Version #1.30
    CURRENT APPLICATION DATA:
      APPLICATION NUMBER: US/08/632,470
       FILING DATE: 08-JUL-1996
      CLASSIFICATION: 435
    ATTORNEY/AGENT INFORMATION:
      NAME: BERRIDGE, WILLIAM P
       REGISTRATION NUMBER: 30,024
      REFERENCE/DOCKET NUMBER: WPB 38238
    TELECOMMUNICATION INFORMATION:
      TELEPHONE: (703)-836-6400
       TELEFAX: (703)-836-2787
  INFORMATION FOR SEQ ID NO:
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       LENGTH: 1484 base pairs
       TYPE: nucleic acid
       STRANDEDNESS: single
       TOPOLOGY: linear
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ACCESSION
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           samples.
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AUTHORS Mossoc. Green, D.H., Perez, B., Velasco, A., Henriquez, R. and
McKenzie, J.D.
  TITLE
           Intracellular bacteria associated with the ascidian Ecteinascidia
           turbinata: Phylogenetic and in situ hybridisation analysis
  JOURNAL
           Mar. Biol. 143, 99-110 (2003)
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           Perez, B., Velasco, A., Henriquez, R. and Sanchez-Puelles, J.M.
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  JOURNAL
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           Direct Submission
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           Submitted (30-AUG-2001) Biotechnology, Pharma Mar S.A., c/Calera 3,
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              0 S L6 AND L16
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L20
              0 S L12 AND L16
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COST IN U.S. DOLLARS

FULL ESTIMATED COST

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TOTAL

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FILE 'LIFESCI' ENTERED AT 10:12:22 ON 12 SEP 2006 COPYRIGHT (C) 2006 Cambridge Scientific Abstracts (CSA)

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ANSWER 1 OF 6 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2006:148843 BIOSIS ACCESSION NUMBER: PREV200600150476 DOCUMENT NUMBER:

Microbial community dynamics during start-up of TITLE:

bio-hydrogen producing reactor.

Ren, N. Q. [Reprint Author]; Xing, D. F.; Gong, M. L.; Li, AUTHOR (S):

Q. B.

Harbin Inst Technol, Sch Municipal and Environm Engn, CORPORATE SOURCE:

> Harbin 150090, Peoples R China rng@hti.edu.cn; ixdf@yahoo.com.cn

Wang, XC [Editor]; Chen, R [Editor]. (2005) pp. 161-168. SOURCE:

Future of Urban Wastewater Systems - Decentralisation and

Reuse.

Publisher: CHINA ARCHITECTURE & BUILDING PRESS,

BAIWANZHUANG XICHENGGU, BEIJING, 10037, PEOPLES R CHINA. Meeting Info.: Conference of the International-Water-

Association (IWA). Xian, PEOPLES R CHINA. May 18 -20, 2005.

Xian Univ Architecture & Technol; Int Water Assoc.

ISBN: 7-112-07386-3(S). Book; (Book Chapter)

Conference; (Meeting) LANGUAGE: English

DOCUMENT TYPE:

ENTRY DATE: Entered STN: 1 Mar 2006

Last Updated on STN: 1 Mar 2006

A laboratory-scale continuous hydrogen production is carried on by using AB molasses as its feed. During these 35 days of the start-up period, microbial community dynamics are monitored by using the denaturing gradient gel electrophoresis (DGGE) analysis of the V3 region of 16S rRNA genes. After running 7-14 days, there is an obvious change in the dominant populations of the microbial community. And this change accompanies with a change of fermentation type. The dominant populations are as follows low G+C gram-positive bacteria, Bacteroides, Actinobacteria and y-Proteobacteria, respectively. Most of the hydrogen producing bacteria are Clostridium sp., Bacteroides sp., Acetanaerobacterium sp., Megasphaera sp., Bacteroides sp., and Enterobacter sp., respectively. The community structure composition and the amount of dominant population are different when the reactor operates in different periods. Different niche conditions lead to different enrichment of bacteria, and forms different microbial community structures.

ANSWER 2 OF 6 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN L9

ACCESSION NUMBER: DOCUMENT NUMBER:

2005:225133 BIOSIS PREV200510010159

TITLE:

Coexistence of bacterial sulfide oxidizers, sulfate

reducers, and spirochetes in a gutless worm (Oligochaeta)

from the Peru margin.

AUTHOR(S):

Blazejak, Anna; Erseus, Christer; Amann, Rudolf; Dubilier,

Nicole [Reprint Author]

CORPORATE SOURCE:

Max Planck Inst Marine Microbiol, Celsiusstr 1, D-28359

Bremen, Germany

ndubilie@mpi-bremen.de

SOURCE:

Applied and Environmental Microbiology, (MAR 2005) Vol. 71,

No. 3, pp. 1553-1561.

CODEN: AEMIDF. ISSN: 0099-2240.

DOCUMENT TYPE:

Article English

LANGUAGE: ENTRY DATE:

Entered STN: 16 Jun 2005

Last Updated on STN: 16 Jun 2005

Olavius crassitunicatus is a small symbiont-bearing worm that occurs at AB high abundance in oxygen-deficient sediments in the East Pacific Ocean. Using comparative 16S rRNA sequence analysis and fluorescence in situ hybridization, we examined the diversity and phylogeny of bacterial symbionts in two geographically distant O. crassitunicatus populations (separated by 385 km) on the Peru margin (water depth, similar to 300 m). Five distinct bacterial phylotypes co-occurred in all specimens from both sites: two members of the gamma-Proteobacteria (Gamma 1 and 2 symbionts), two members of the delta-Proteobacteria (Delta I and 2 symbionts), and one spirochete. A sixth phylotype belonging to the delta-Proteobacteria (Delta 3 symbiont) was found in only one of the two host populations.

Three of the O. crassitunicatus bacterial phylotypes are closely related to symbionts of other gutless oligochaete species; the Gamma 1 phylotype is closely related to sulfide-oxidizing symbionts of Olavius algarvensis, Olavius loisae, and Inanidrilus leukodermatus, the Delta 1 phylotype is closely related to sulfate-reducing symbionts of O . algarvensis, and the spirochete is closely related to spirochetal symbionts of O. loisae. contrast, the Gamma 2 phylotype and the Delta 2 and 3 phylotypes belong to novel lineages that are not related to other bacterial symbionts. phylogenetically diverse yet highly specific and stable association in which multiple bacterial phylotypes coexist within a single host has not been described previously for marine invertebrates.

ANSWER 3 OF 6 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

2005:253069 BIOSIS ACCESSION NUMBER: PREV200510037051 DOCUMENT NUMBER:

Bacterial community shift along a subsurface geothermal TITLE:

water stream in a Japanese gold mine.

Hirayama, Hisako [Reprint Author]; Takai, Ken; Inagaki, AUTHOR (S):

Fumio; Yamato, Yu; Suzuki, Masae; Nealson, Kenneth H.;

Horikoshi, Koki

Japan Agcy Marine Earth Sci and Technol, Extremobiosphere CORPORATE SOURCE:

Res Ctr, Subground Animalcule Retrieval SUGAR Project, 2-15

Natsushima Cho, Yokosuka, Kanagawa 2370061, Japan

hirayamah@jamstec.go.jp

Extremophiles, (APR 2005) Vol. 9, No. 2, pp. 169-184. SOURCE:

ISSN: 1431-0651.

DOCUMENT TYPE: Article English LANGUAGE:

Entered STN: 8 Jul 2005 ENTRY DATE:

Last Updated on STN: 8 Jul 2005

Change of bacterial community occurring along a hot water stream in the AB Hishikari gold mine, Japan, was investigated by applying a combination of various culture-independent techniques. The stream, which is derived from a subsurface anaerobic aquifer containing plentiful CO2, CH4, H-2, and NH4+, emerges in a mine tunnel 320 m below the surface providing nutrients for a lush microbial community that extends to a distance of approximately 7 m in the absence of sunlight-irradiation. Over this distance, the temperature decreases from 69 degrees C to 55 degrees C, and the oxidation-reduction potential increases from - 130 mV to + 59 mV. hot upper reaches of the stream, the dominant phylotypes were: 1) a deeply branching lineage of thermophilic methane-oxidizing gamma-Proteobacteria, and 2) a thermophilic hydrogen- and sulfur-oxidizing Sulfurihydrogenibium In contrast, the prevailing phylotypes in the middle and lower parts of the stream were closely related to ammonia-oxidizing Nitrosomonas and nitrite-oxidizing Nitrospira spp.. Changes in the microbial metabolic potential estimated by competitive PCR analysis of genes encoding the enzymes, particulate methane monooxygenase (pmoA), ammonia monooxygenase (amoA), and putative nitrite oxidoreductase (norB), also substantiated the community shift indicated by 16S rRNA gene analysis. The diversity of putative norB lineages was assessed for the first time in the hot water environment. Estimation of dominant phylotypes by whole-cell fluorescent in situ hybridization and changes in inorganic nitrogen compounds such as decreasing ammonium and increasing nitrite and nitrate in the mat-interstitial water along the stream were consistent with the observed transition of the bacterial community structure in the stream.

BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN ANSWER 4 OF 6 L9 2002:475652 BIOSIS ACCESSION NUMBER:

DOCUMENT NUMBER: PREV200200475652

Marine nematodes as hosts for chemoautotrophic symbionts. TITLE: Ott, Joerg [Reprint author]; Bright, Monika [Reprint author]; Nussbaumer, Andrea [Reprint author]; Vanura, Katrina; Eichinger, Irmgard [Reprint author]; Schabussova, AUTHOR (S):

Irma [Reprint author]

CORPORATE SOURCE: Institute of Ecology and Conservation Biology, University

of Vienna, Althanstrasse 14, A-1090, Vienna, Austria

joerg.ott@univie.ac.at

SOURCE: Nematology, (2002) Vol. 4, No. 2, pp. 187-188. print.

Meeting Info.: Fourth International Congress of Nematology Programme and Abstracts. Tenerife, Canary Islands, Spain.

June 08-13, 2002. ISSN: 1388-5545.

DOCUMENT TYPE: Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)

LANGUAGE:

English

ENTRY DATE:

Entered STN: 11 Sep 2002

Last Updated on STN: 11 Sep 2002

L9 ANSWER 5 OF 6
ACCESSION NUMBER:

BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN 1999:126171 BIOSIS

DOCUMENT NUMBER:

PREV199900126171

TITLE:

Bacteria of the genus Roseobacter associated with the toxic

dinoflagellate Prorocentrum lima.

AUTHOR (S):

Prokic, Ivo; Bruemmer, Franz; Brigge, Theo; Goertz, Hans D.; Gerdts, Gunnar; Schuett, Christian; Elbraechter, Malte;

Mueller, Werner E. G. [Reprint author]

CORPORATE SOURCE:

Inst. Physiol. Chem., Abt. Angewandte Mol., Univ.,

Duesbergweg 6, D-55099 Mainz, Germany

SOURCE:

Protist, (Dec., 1998) Vol. 149, No. 4, pp. 347-357. print.

ISSN: 1434-4610.

DOCUMENT TYPE:

Article English

LANGUAGE: ENTRY DATE:

Entered STN: 17 Mar 1999

Last Updated on STN: 17 Mar 1999

The dinoflagellate Prorocentrum lima is known to produce diarrhetic AB shellfish poisons. However, it is yet unclear if the dinoflagellates themselves or the bacteria associated with them produce the toxins. Here we analyze the toxicity as well as the spectrum of bacteria in two cultures of P. lima, namely P. lima-SY and P. lima-ST, which initially derived from the same P. lima strain PL2V. Toxicity tests, applying the Artemia bioassay revealed in both cultures high levels of toxins. The bacteria, associated with the two cultures, were identified by PCR/nucleotide sequence analysis of the 16S rRNA gene. From cultures of P. lima-SY the dominant sequence was found to share a 93.7% similarity with the sequence of Roseobacter algocolus (R. algicola); the relative abundance was determined to be 83%. In addition three further sequences of bacteria, grouped to the alpha-Protobacteria have been identified: Paracoccus denitrificans (90.8%), R. algocolus (94.4%) and Rhizobium huakuii (92.6%). The identification of bacteria in P. lima-ST revealed that most share highest similarity with Bartonella taylorii but with a relatively low score of 87%. In addition to this sequence, two sequences with high similarity to the genus Roseobacter were obtained. The other sequences identified have not been detected in P. lima-SY. Studies with pure bacterial strains, previously isolated from a culture of P. lima-ST and subsequently cultured on agar plates, revealed that none of them was identical to those identified in the dinoflagellate culture itself. An explanation for the change of the spectrum of bacteria in the different cultures can only be expected when axenic cultures from P. lima are available.

L9 ANSWER 6 OF 6 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN ACCESSION NUMBER: 1998:313104 BIOSIS

ACCESSION NUMBER: DOCUMENT NUMBER:

PREV199800313104

TITLE:

Alcanivorax borkumensis gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine

bacterium.

AUTHOR(S):

Yakimov, Michail M.; Golyshin, Peter N. [Reprint author]; Lang, Siegmund; Moore, Edward R. B.; Abraham, Wolf-Rainer; Luensdorf, Heinrich; Timmis, Kenneth N.

CORPORATE SOURCE: Div. Microbiol., GBF Natl. Res., Cent. Biotechnol.,

Mascheroder Weg 1, D-38124 Braunschweig, Germany

International Journal of Systematic Bacteriology, (April, SOURCE:

1998) Vol. 48, No. 2, pp. 339-348. print.

CODEN: IJSBA8. ISSN: 0020-7713.

DOCUMENT TYPE: Article LANGUAGE: English

Entered STN: 22 Jul 1998 ENTRY DATE:

Last Updated on STN: 22 Jul 1998

During screening for biosurfactant-producing, n-alkane-degrading marine bacteria, six heterotrophic bacterial strains were isolated from enriched mixed cultures, obtained from sea water/sediment samples collected near the Isle of Borkum (North Sea), using Mihagol-S (C14,15-n-alkanes) as principal carbon source. These Gram-negative, aerobic, rod-shaped bacteria use a limited number of organic compounds, including aliphatic hydrocarbons, volatile fatty acids, and pyruvate and its methyl ether. During cultivation on n-alkanes as sole source of carbon and energy, all strains produced both extracellular and cell-bound surface-active glucose lipids which reduced the surface tension of water from 72 to 29 mN m-1 (16). This novel class of glycolipids was found to be produced only by these strains. The 16S rRNA gene sequence analysis showed that these strains are all members of the gamma-subclass of the Proteobacteria. Their phospholipid ester-linked fatty acid composition was shown to be similar to that of members of the genus Halomonas, although they did not demonstrate a close phylogenetic relationship to any previously described species. On the basis of the information summarized above, a new genus and species, Alcanivorax borkumensis, is described to include these bacteria. Strain SK2T is the type strain of A. borkumensis.

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(FILE 'HOME' ENTERED AT 10:11:52 ON 12 SEP 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:12:22 ON 12 SEP 2006

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0 S S GAMMA (A) PROTOBACTERI? L2

1 S GAMMA (A) PROTOBACTERIUM

6 S GAMMA (A) PROTOBACTERI? L4 L5

0 S 16S(W) RIBOZOMAL (W)RNA

13711 S 16S(W) RIBOSOMAL (W)RNA

85 S PROTOBACTERI? L7

L8 6 S L6 AND L7

6 DUP REM L8 (0 DUPLICATES REMOVED) L9

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429 ECTEINASCIDIA (W) TURBINATA

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ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN L11

2003:455344 BIOSIS AN

PREV200300455344 DN

Intracellular bacteria associated with the ascidian Ecteinascidia ΤI turbinata: Phylogenetic and in situ hybridisation analysis.

Moss, C. [Reprint Author]; Green, D. H.; Perez, B.; Velasco, A.; ΑU Henriquez, R.; McKenzie, J. D.

Marine Resource Centre, Integrin Advanced Biosystems, Barcaldine, Oban, CS Argyll, PA37 1SE, UK

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claire moss@yahoo.com
     Marine Biology (Berlin), (July 2003) Vol. 143, No. 1, pp. 99-110. print.
SO
     CODEN: MBIOAJ. ISSN: 0025-3162.
DT
     Article
LA
     English
     Entered STN: 1 Oct 2003
ED
     Last Updated on STN: 1 Oct 2003
     The ascidian Ecteinascidia turbinata (Herdman) is a
ΑB
     colonial sea squirt found in the Caribbean and Mediterranean Seas. In the
     present study, the bacterial complement of E. turbinata has been assessed
     by 16S rRNA gene analysis and the most commonly occurring strains
     identified by restriction fragment length polymorphism. Three strains
     were found to predominate using this approach, with one representing >50%
     of clones from both larval and adult material. The 16S rRNA gene sequence
     of the most commonly occurring strain did not match with any known
     bacterial sequences and could only be assigned to the gamma-proteobacteria
     subdivision. The two other frequently occurring strains were assigned to
                     In situ hybridisation analysis with eubacterial probes to
     the Mollicutes.
     16S rRNA revealed the presence of apparently endosymbiotic bacteria in
     adult and larval tissue, and electron microscopy confirmed the presence of
     putative bacteriocytes in the larval tissue. The presence of the same
     bacteria in the brooded larvae suggested that they were vertically
     transmitted from parent to offspring. Further hybridisation using a novel
     probe designed to be specific to the 16S rRNA sequence of the dominant
     strain, highlighted the same cell types as that revealed by the
     eubacterial probe. The results suggest that the bacteria represent a
     novel strain, denoted "Candidatus Endoecteinascidia frumentensis", and
     that they may have an important role in the biology of E. turbinata.
     Ecology: environmental biology - Animal
                                               07508
CC
     Ecology: environmental biology - Oceanography
ΤТ
     Major Concepts
        Marine Ecology (Ecology, Environmental Sciences)
     Chemicals & Biochemicals
IT
          16S ribosomal RNA
IT
     Methods & Equipment
        in situ hybridization analysis: laboratory techniques; phylogenetic
        analysis: mathematical and computer techniques
     Caribbean (West Indies, Neotropical region); Mediterranean Sea (North
GT
     Atlantic, Atlantic Ocean)
ORGN Classifier
        Urochordata
                      85104
     Super Taxa
        Protochordata; Chordata; Animalia
     Organism Name
          Ecteinascidia turbinata (species)
     Taxa Notes
        Animals, Chordates, Invertebrates, Protochordates
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L13 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:455344 BIOSIS
DOCUMENT NUMBER:
                    PREV200300455344
                    Intracellular bacteria associated with the ascidian
TITLE:
                    Ecteinascidia turbinata: Phylogenetic and in situ
                    hybridisation analysis.
                    Moss, C. [Reprint Author]; Green, D. H.; Perez, B.;
AUTHOR(S):
                    Velasco, A.; Henriquez, R.; McKenzie, J. D.
                    Marine Resource Centre, Integrin Advanced Biosystems,
CORPORATE SOURCE:
                    Barcaldine, Oban, Argyll, PA37 1SE, UK
                    claire_moss@yahoo.com
                    Marine Biology (Berlin), (July 2003) Vol. 143, No. 1, pp.
SOURCE:
                    99-110. print.
                    CODEN: MBIOAJ. ISSN: 0025-3162.
DOCUMENT TYPE:
                    Article
LANGUAGE:
                    English
                    Entered STN: 1 Oct 2003
ENTRY DATE:
                    Last Updated on STN: 1 Oct 2003
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AB
     found in the Caribbean and Mediterranean Seas. In the present study, the
     bacterial complement of E. turbinata has been assessed by 16S rRNA gene
     analysis and the most commonly occurring strains identified by restriction
     fragment length polymorphism. Three strains were found to predominate
     using this approach, with one representing >50% of clones from both larval
     and adult material. The 16S rRNA gene sequence of the most commonly
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     could only be assigned to the gamma-proteobacteria subdivision. The two
     other frequently occurring strains were assigned to the Mollicutes. In
     situ hybridisation analysis with eubacterial probes to 16S rRNA revealed
     the presence of apparently endosymbiotic bacteria in adult and larval
     tissue, and electron microscopy confirmed the presence of putative
     bacteriocytes in the larval tissue. The presence of the same bacteria in
     the brooded larvae suggested that they were vertically transmitted from
     parent to offspring. Further hybridisation using a novel probe designed
     to be specific to the 16S rRNA sequence of the dominant strain,
     highlighted the same cell types as that revealed by the eubacterial probe.
     The results suggest that the bacteria represent a novel strain, denoted "
     Candidatus Endoecteinascidia frumentensis",
     and that they may have an important role in the biology of E. turbinata.
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L19			L6 AND L16					
L20	0	S	L12 AND L16					•

	Issue Date	Page 8	Document ID	Title
			ບຣ	Sequences from an
1	20060727	22	2006016722	endosymbiont and
			8 A1	their uses

	Issue Date	Page s	Document	Title
1	20060706		7006017745	System for detecting polynucleotides
2	20051110		US	Development of DNA probes and immunological reagents specific for cell surface-expressed molecules and transformation-associated genes
3	20050728	75	US 2005016370 9 A1	Development of DNA probes and immunological reagents specific for cell surface-expressed molecules and transformation-associated genes
4	20020829	119	US 2002012011 6 Al	ENTEROCOCCUS FAECALIS POLYNUCLEOTIDES AND POLYPEPTIDES
5	20041102	73	US 6811972 B1	Development of DNA probes and immunological reagents specific for cell surface-expressed molecules and transformation-associated genes
6	20040420	59	US 6723837 B1	Nucleic acid molecule and encoded protein associated with sterol synthesis and metabolism

	Issue	Page	Document		Title	
	Date	8	s II		•	
7	20010710	a a	US 62 B1	258359	Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides	
8	20010619	וסו	US 62 B1	248330	Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides	
9	20001226	30	US 6: A	165992	Histidine kinase	
10	19981201	92	US 5	042460	Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides	
11	19970311	16	US 5	<i>6</i> 10060	Isolated Helicobacter hepaticus	
12	19961210	12	US 5	582975	Nucleic acid probes to staphylococcus aureus	

	L #	Hits	Search Text
1	L1	1	"5976791".pn.
2	L2	26	candidatus
3	L3	0	l1 and 12
4	L4	900	16S adj ribosomal adj RNA
5	L5	51	Ecteinascidia adj turbinata
6	L6	0	14 same 15
7	L7	1	Candidatus adj endoecteinascidia adj frumentensis
8	L8	1	gamma adj protobacteri\$3
9	L9		ESTEBAN IGLESIS MORENO PEREZ
10	L11	0	15 and 110
11	L10	12	14 and 19